observation that irritants, which do not induce IL-10 expression, are not effective in AA, whereas contact allergens such as DCP are [6]. On the other hand, phototherapy may be beneficial in AA. Ultraviolet light has been shown to be a potent inducer of IL-10 expression in keratinocytes. Theoretically, intralesional application of recombinant IL-10 should provide a novel therapeutic approach for AA. This approach would be more specific than application of a potent contact allergen.

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# An Allele of the Interleukin-1 Receptor Antagonist as a Genetic Severity Factor in Alopecia Areata

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Alopecia areata is a common inflammatory disorder affecting the hair and, in some cases, the nails. There is a strong hereditary component with a positive family history ranging from 10% to 25% in different studies, although the true figure may be higher, as mild cases are often overlooked. Associations with several major histocompatibility complex loci have been reported including DR-4 and DQW-7.

We have been investigating the question "why do some patients develop one patch of alopecia areata that regrows without further episodes, whereas others develop alopecia totalis or universalis for the rest of their lives?" Perhaps the answer lies in genes that determine severity rather than susceptibility to alopecia areata.

One of the major determinants of an inflammatory response in the skin is the local production of pro-inflammatory cytokines and their antagonists, in particular, interleukin-1 (IL-1) and the IL-1 receptor antagonist (IL-1ra). If an individual produces high levels of pro-inflammatory cytokines and/or low levels of cytokine inhibitor, this would result in the amplification and perpetuation of an inflammatory response to any stimulus. We postulated that polymorphisms within cytokine and related genes may be associated with alopecia areata and other inflammatory skin diseases. These polymorphisms could result in differences in cytokine/cytokine inhibitor production that would favor the inflammatory response.

There are very high levels of interleukin-1$\alpha$ (IL-1$\alpha$) sequestered inside normal keratinocytes that can be released following trauma, ultraviolet B irradiation and local infection. We have previously demonstrated that there are decreased levels of the endogenous antagonist to IL-1, the IL-1 receptor antagonist (IL-1ra) in other inflammatory skin diseases including psoriatic plaques. IL-1$\alpha$ inhibits human hair-follicle growth and hair-fiber production in whole organ cultures [1]. IL-1 may therefore play a role in alopecia areata through a direct growth-inhibitory effect on hair follicles. The gene for IL-1ra (IL-1rn) is located on the long arm of chromosome 2 on a 430-kb stretch of DNA that also contains the genes for IL-1$\alpha$ and IL-1$\beta$.

We have described a variable number tandem repeat polymorphism (VNTR) in intron 2 of the IL-1 ra gene. Five alleles of the system were identified corresponding to two, three, four, five, and six copies of an 86–base pair repeat sequence. We have tested the genetic association of alopecia areata with this IL-1ra polymorphism by comparing the allele frequencies in patients with alopecia areata and healthy individuals in a case-control association study.

## MATERIALS AND METHODS

Blood was collected from 261 unrelated, healthy Caucasian individuals from the North of England population and from 90 alopecia areata patients attending the dermatology out-patient clinic at the Royal Hallamshire Hospital in Sheffield. Prior to genetic analysis, the patients were divided into three groups: patchy alopecia, alopecia totalis, and alopecia universalis. DNA was extracted using standard methods.

Polymorphism typing was based on a PCR method as previously described [2]. The rate of gene carriage (i.e., the number of individuals carrying at least one copy of a specific allele as a proportion of the total number of individuals) was calculated.

## RESULTS

The carriage of allele 2 of the interleukin-1 receptor antagonist gene is shown in **Fig 1**. This was 41% in the general population compared with 44% in patients with patchy alopecia areata, 66% in patients with alopecia totalis, and 77% in alopecia universalis ($p = 0.005$, OR = 5).

## DISCUSSION

Alopecia areata seems to be a polygenic disease with genetic susceptibility and severity factors. Genetic factors probably interact with environmental factors such as infection to trigger the disease. The severity of an inflammatory response to an environmental trigger may be determined by the balance of pro-inflammatory (e.g., IL-1$\alpha$, IL-1$\beta$) and anti-inflammatory cytokines and/or cyto-

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Abbreviation: VNTR, variable number tandem repeat polymorphism.

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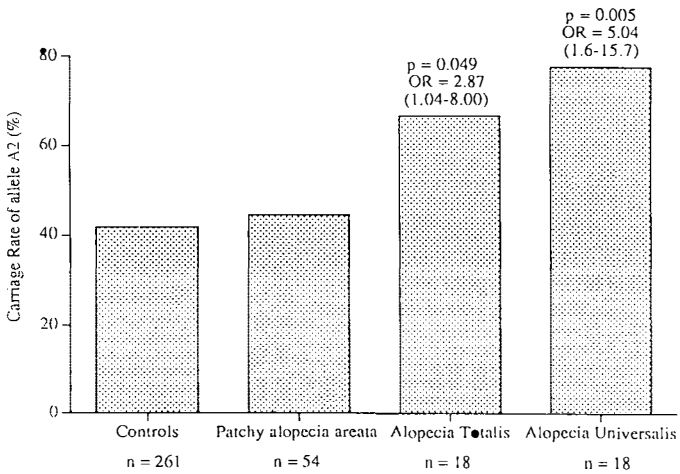


**Figure 1. Carriage rate of the allele 2 in the healthy control population compared with patients with patchy alopecia areata, alopecia totalis, and alopecia universalis**. The chi-squared p value between the controls and the alopecia totalis group is 0.049 with an odds ratio of 2.87 with 95% confidence limits of 1.04 to 8.00. The p value between the controls and the alopecia universalis group is 0.005 and the odds ratio is 5.04 with 95% confidence limits of 1.60 to 15.7. Individuals in patient groups and the healthy population were unrelated, white Caucasians matched for age.

kine inhibitors (IL-1ra). A relative deficiency of the IL-1ra could result in the perpetuation and increased severity of chronic inflammatory skin diseases.

We have demonstrated that allele 2 of a VNTR polymorphism in the interleukin-1 receptor antagonist gene is significantly increased in patients with alopecia totalis and alopecia universalis. This suggests that allele 2 is not involved in the susceptibility to alopecia areata but affects the severity or extent of the disease.

We have also demonstrated an association between allele 2 of this IL-1ra polymorphism and the severity of other inflammatory diseases of epithelial tissues, including systemic lupus erythematosus, lichen sclerosis, and inflammatory bowel disease [3–5].

We have tested several other polymorphisms on chromosome 2 and have shown that the strongest association with alopecia areata is with the IL-1 receptor antagonist gene locus. We are currently investigating the VNTR polymorphism in intron 2 of the IL-1ra gene to determine if it is associated with a specific production phenotype for either the secreted or intracellular form of the interleukin-1 receptor antagonist. It is probable that this polymorphism is a marker for a functional polymorphism elsewhere in the IL-1rn gene or a nearby gene.

We report an association of an immunoregulatory gene with the clinical severity of alopecia areata. Our observation that the same allele of this polymorphism is associated with severity in several other inflammatory diseases suggests that the IL-1rn gene is a general modifying factor in chronic inflammatory diseases of epithelial, and possibly other, tissues.

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# C3H/HeJ Mouse Model for Alopecia Areata

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Human alopecia areata is a relatively common disease characterized by alopecia of acute onset that is focal, diffuse, or combinations of the two. Microscopically, this non-scarring human alopecia consists of dystrophic anagen or telogen hair follicles accompanied by a mononuclear cell infiltrate in and around hair follicles [1,2]. Comparable diseases have been sporadically reported in Dachshund and Miniature Poodle dogs [3,4], Siamese cats [3], Palomino and Appaloosa horses [3], and some non-human primate species [3]. A spontaneous mutation occurred in the rat that has been developed as an animal model for alopecia areata. The mutant stock has been designated the Dundee Experimental Bald Rat [5].

A large number of spontaneous and induced mutations with alopecia as a major part of their phenotype are described and most are available through national repositories, such as the one at The Jackson Laboratory. Although initial review of the literature and case materials failed to identify a mouse model for alopecia areata, in 1991 a single C3H/HeJ female mouse from a large production colony was diagnosed with what appeared to be alopecia areata. Subsequent investigations have confirmed that alopecia areata occurs spontaneously as an aging disease of very low frequency (0.25% of female mice, 0.035% of male mice 5–6 months of age) in this strain.

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